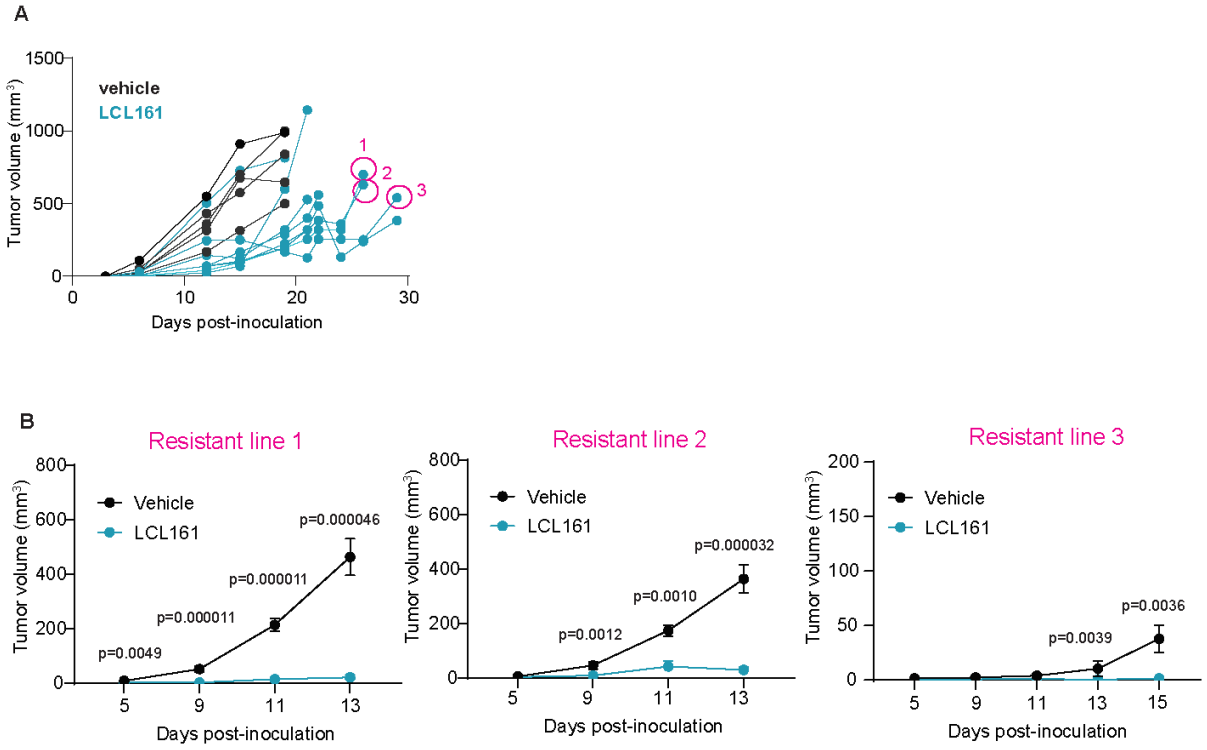

Fig. S1. **Resistance to cIAP1/2 antagonism is tumor cell extrinsic.** (**A**) C57BL/6 mice were inoculated with $2.5x10^5$ 6694c2 cells expressing yellow fluorescent protein (YFP) subcutaneously. Mice were treated with vehicle or 75mg/kg LCL161 every 3 days starting on day 3. Individual tumor growth curves are shown. The tumors indicated in pink circles were harvested, digested, and plated into cell culture. After 7 days of culture, tumor cell lines were sorted for YFP^+ tumor cells to remove fibroblast contamination. (**B**) Cell lines derived from resistant tumors in (A) were reimplanted into C57BL/6 mice ($2.5x10^5$ cells per mouse subcutaneously), and mice were treated every 3 days starting on day 3 by oral gavage with LCL161 (75 mg/kg) or vehicle control (sodium acetate. Lines 1 and 2 responded to LCL161 equivalently to the parental 6694c2 cell line. Line 3 exhibited slower growth kinetics in both vehicle- and LCL161-treated mice. All data are shown with n=10 animals per group. Data are presented as mean ± SEM in (B) and p-values were calculated by Mann-Whitney test with two-stage step up false discovery rate. cIAP1/2, cellular inhibitor of apoptosis proteins 1/2.

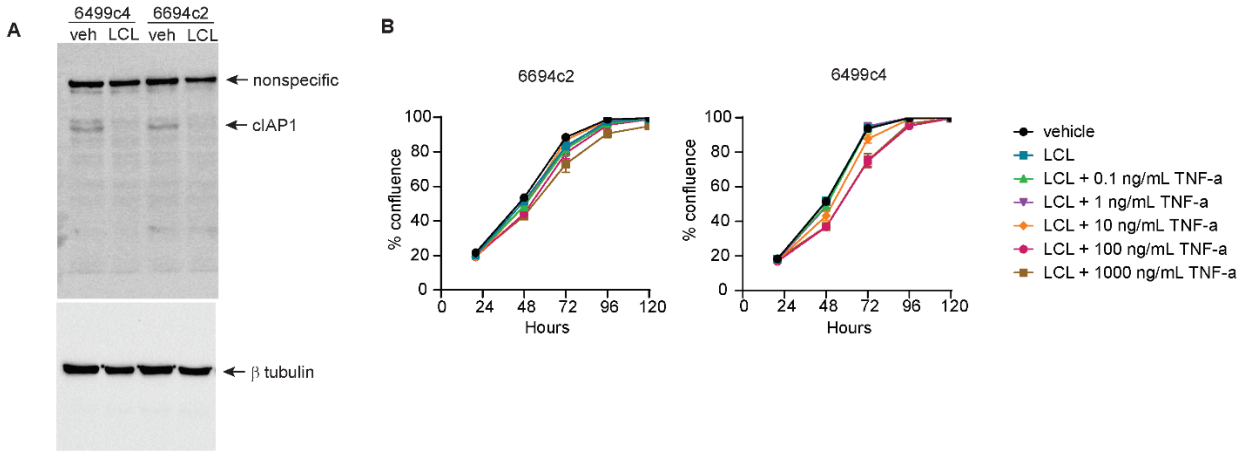

Fig. S2. Pancreatic tumor cell lines are resistant to cIAP1/2 antagonism and TNF-α in vitro. (**A**) PDAC cell lines 6694c2 and 6499c4 were cultured with DMSO or 500 nM LCL161 for 24 hours. Protein lysates were collected and analyzed by SDS-PAGE and immunoblotting for cIAP1 or beta-tubulin. (**B**) PDAC cell lines 6694c2 and 6499c4 were cultured with 500 nM LCL161 and mouse TNF-α at the indicated concentrations. Confluency was measured by imaging cytometry (Celigo). Data are representative of 4 independent experiments with 3 replicates per group. Data are presented as mean ± SEM in (B). TNF-α, tumor necrosis factor-alpha; PDAC, pancreatic ductal adenocarcinoma.

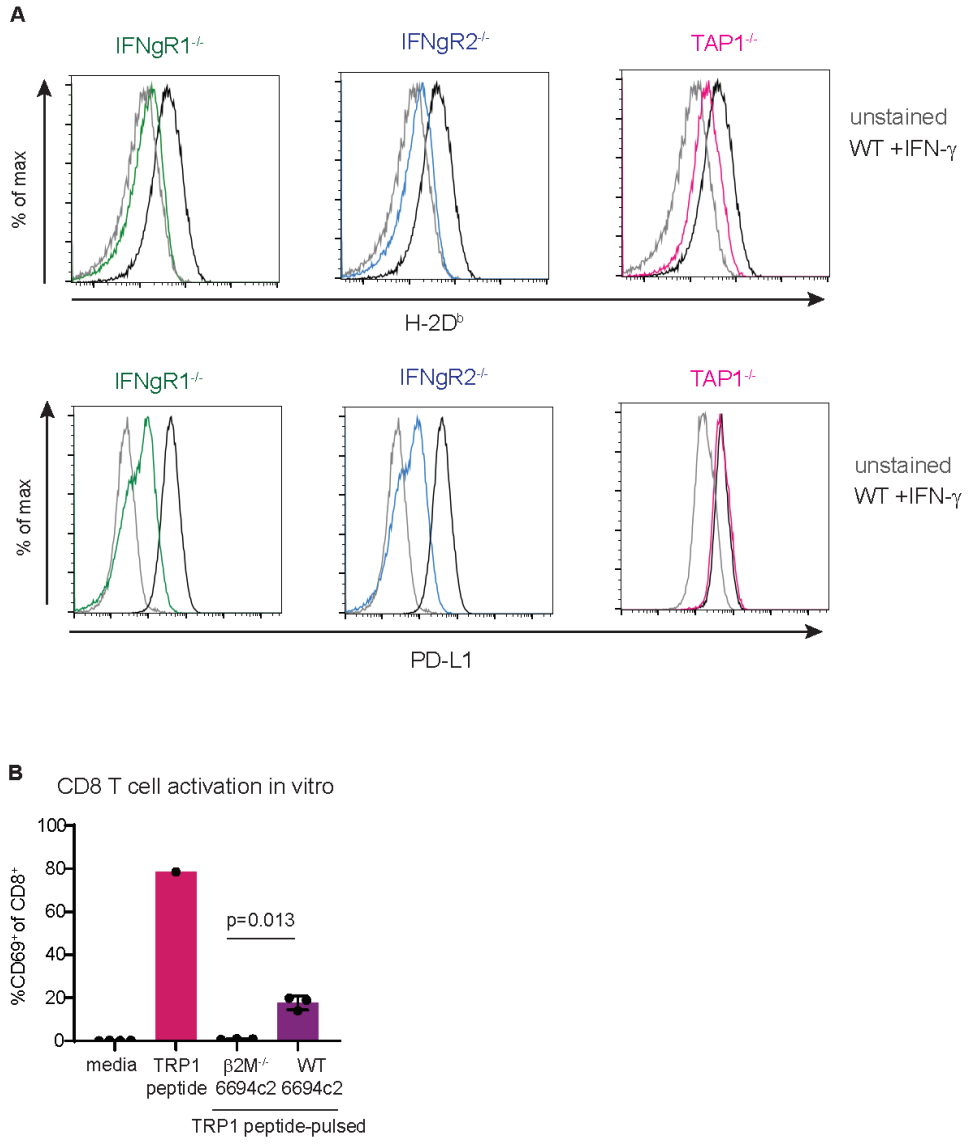

Fig. S3. **Validation of CRISPR/Cas9 genome modified cell lines.** (**A**) 6694c2 cells deficient in the indicated genes were cultured for 24 hours with 100ng/mL IFN-γ. Cells were trypsinized and analyzed by flow cytometry for MHC class I (H-2D^{b}) or PD-L1. Parental 6694c2 cells treated with IFN-γ are shown in black lines as a positive control. (**B**) 6694c2 WT and β2M$^{-/-}$ tumor cells were cultured with IFN-γ and 10nM TRP1 peptide for 24 hours. Cells were trypsinized, washed twice, and replated with CD8 T cells isolated by magnetic beads from spleen and lymph nodes of a TRP1^{high} mouse. CD8 T cell activation was assessed after 18 hours of coculture by flow cytometry for CD69. Incubation of TRP1 CD8 T cells with TRP1 peptide included during the 18 hour culture period (pink bar) was used as a positive control. Data are representative of 3 independent experiments with 3 replicates per group. Data are presented as mean ± SEM (B) and p-values were calculated by Mann-Whitney test. CRISPR, clustered regularly interspaced short palindromic repeats; MHC, major histocompatibility complex; IFN, interferon; TRP1, tyrosinase-related protein 1.

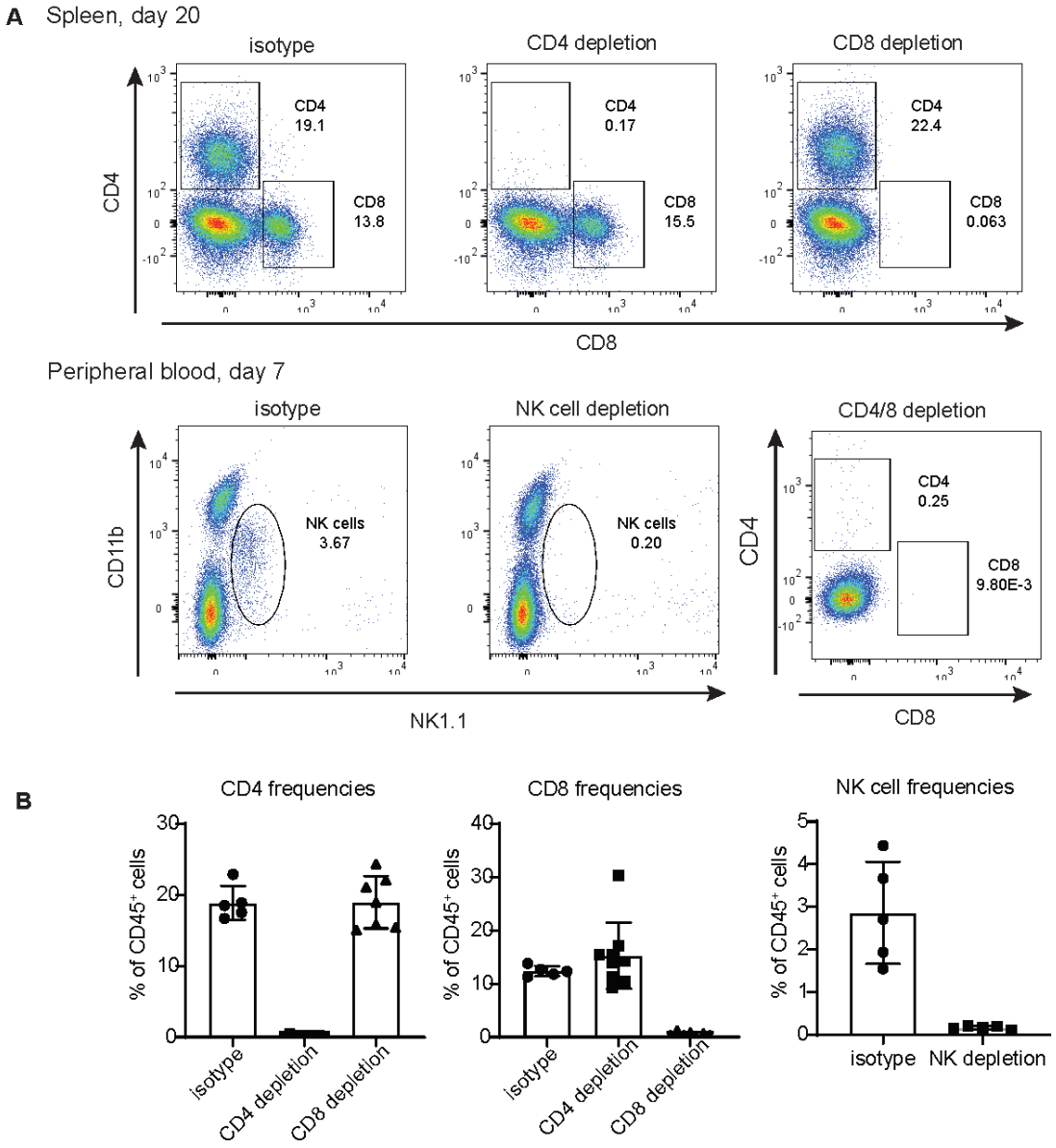

Fig. S4. Confirmation of in vivo cell depletions from Figure2. (**A**) Tumor-bearing mice were treated with depleting antibodies to CD4 (100µg per mouse every 3 days), to CD8 (100µg per mouse every 3 days), to both CD4 and CD8 (100µg per mouse each antibody every 3 days), to NK1.1 (100µg per mouse every 3 days), or with an isotype control antibody as in Fig. 2. Cell depletions were confirmed by flow cytometry of peripheral blood or spleen. (**B**) Quantification of the plots from (A). Data are representative of 2 independent experiments with 5 mice per group. Data are presented as mean ± SD in (B).

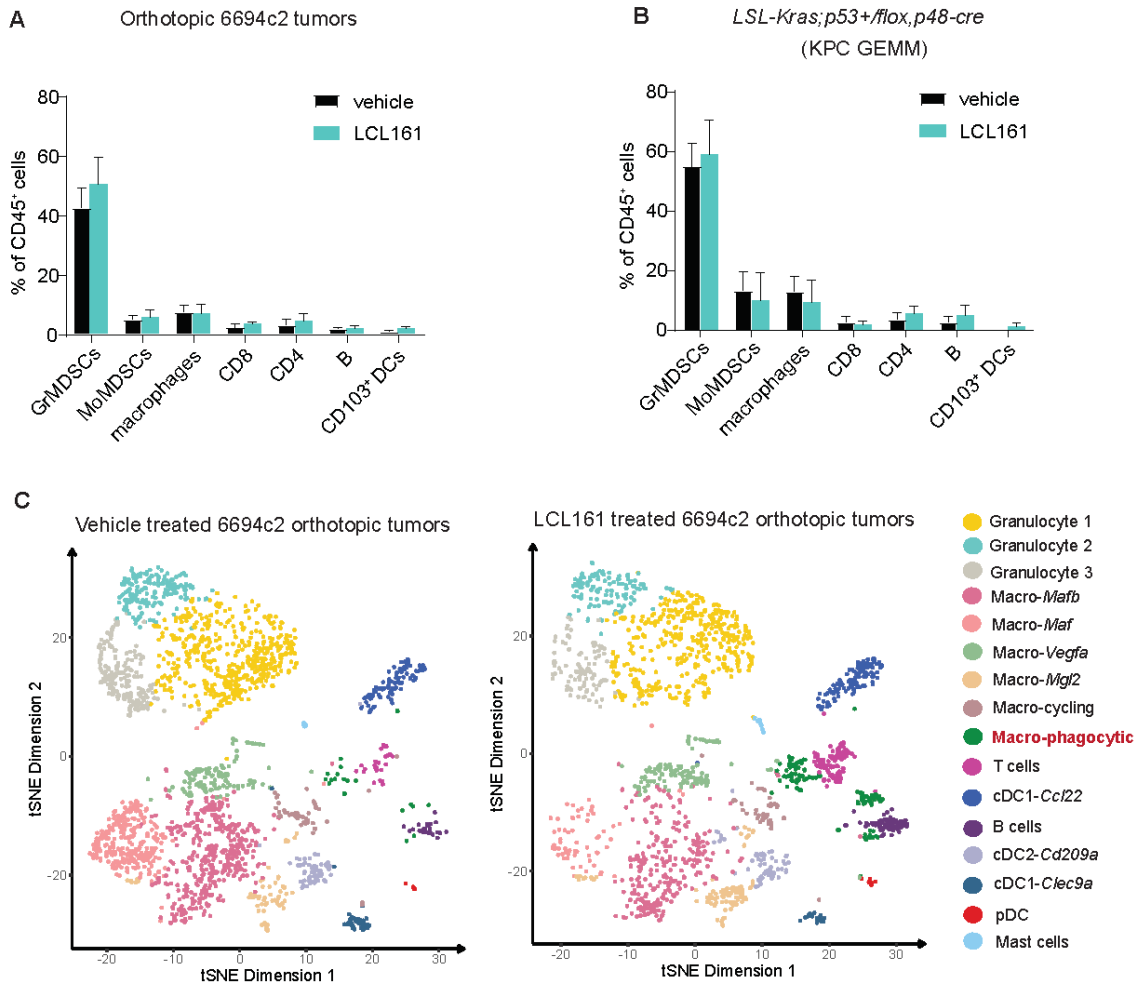

Fig. S5. cIAP1/2 antagonism does not increase T cell accumulation in poorly immunogenic pancreatic cancer models. (**A**) Orthotopic 6694c2 tumors were harvested at day 18 from mice treated with vehicle or LCL161, digested, and analyzed by flow cytometry. Cell types were gated from $CD45^+$ cells and defined as: GrMDSCs ($CD11b^+Gr1^+Ly6C^-$); MoMDSCs ($CD11b^+Gr1^{low}Ly6C^+$); macrophages ($CD11b^+Gr1^-CD11c^-I/A^{b+/-}$); CD8 ($CD11b^-Gr1^-CD8\alpha^+$); CD4 ($CD11b^-Gr1^-CD4^+$); B cells ($CD11b^-Gr1^-B220^+$); $CD103^+$ DCs ($CD11b^-Gr1^-CD11c^+Ly6c^-SiglecF^-CD103^+$). Data are representative of 10 independent experiments with 5 mice per group. (**B**) Tumors at endpoint from KPC GEMM mice from Fig. 1D were digested and analyzed by flow cytometry. Representative of 2 experiments with n=5 mice per group. (**C**) tSNE representation of cells from Fig. 3A separated by treatment condition. Data are presented as mean ± SD in (A) and (B). KPC GEMM, lox-stop-lox-KrasG12D;p53+/flox;p48cre+, GrMDSCs, granulocytic myeloid-derived suppressor cells; MoMDSCs, monocytic myeloid-derived suppressor cells; DCs, dendritic cells; tSNE, t-distributed stochastic neighbor embedding.

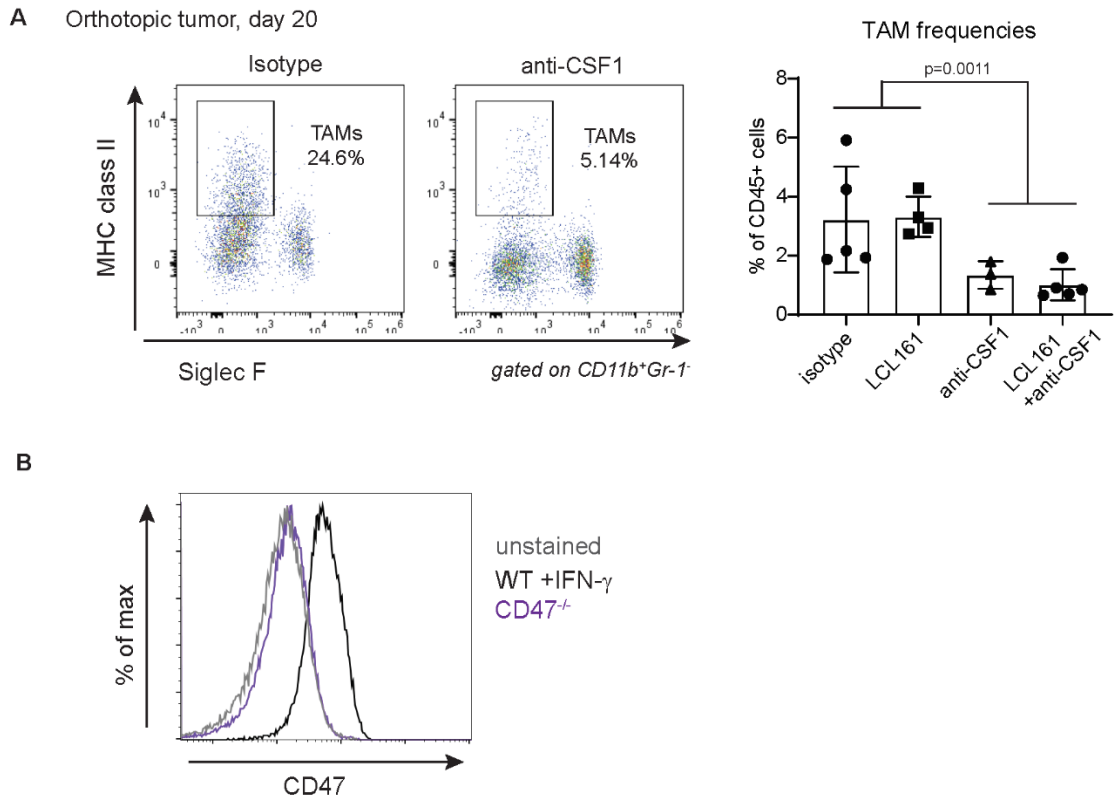

Fig. S6. Confirmation of macrophage depletion and CD47 deficiency from Figure 4. (**A**) Orthotopic 6694c2 tumors from mice treated with LCL161 (75 mg/kg), anti-CSF1 (100 μg per mouse), or a combination of both (from Fig. 4A) were digested and analyzed by flow cytometry. Flow plots are gated on live $CD45^+$ $CD11b^+$ $Gr1^-$ cells comparing tumor-associated macrophage (TAM) frequencies and p-values were calculated by Mann-Whitney test comparing all mice that received anti-CSF1 versus all mice that did not receive anti-CSF1. Results are representative of 2 independent experiments with n=5 mice per group. CSF-1, colony stimulating factor-1. (**B**) 6694c2 wild-type (WT) and $CD47^{-/-}$ cells were cultured for 24 hours with 100ng/mL IFN-γ. Cells were trypsinized and analyzed by flow cytometry for CD47.

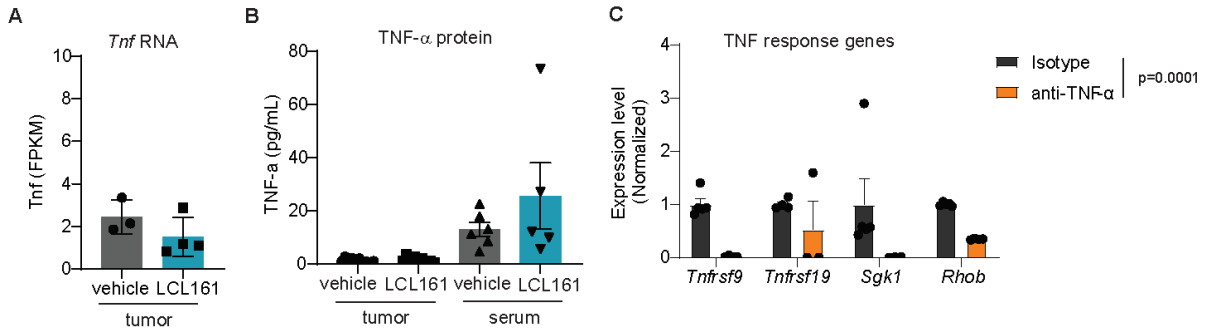

Fig. S7. TNF-α mRNA and protein abundance are low in 6694c2 tumors and not increased with LCL161 treatment. (**A**) Total RNA was extracted from Day 12 orthotopic 6694c2 tumors from mice treated with vehicle or 75mg/kg LCL161 every 3 days. *Tnf* transcripts were quantified by RNAseq. (**B**) Protein lysates from day 12 orthotopic 6694c2 tumors as in (A) were extracted. Serum was collected from the same mice and analyzed by cytokine bead array for TNF-α protein. (**C**) Total RNA was prepared from spleens of mice treated with anti-TNF-α antibodies in Fig. 5D and analyzed by quantitative PCR for the indicated TNF-α response genes. Values are normalized to *Gapdh.* All data are shown, with individual points showing biological replicates. Data are presented as mean ± SEM and p-values were calculated by two-way ANOVA.

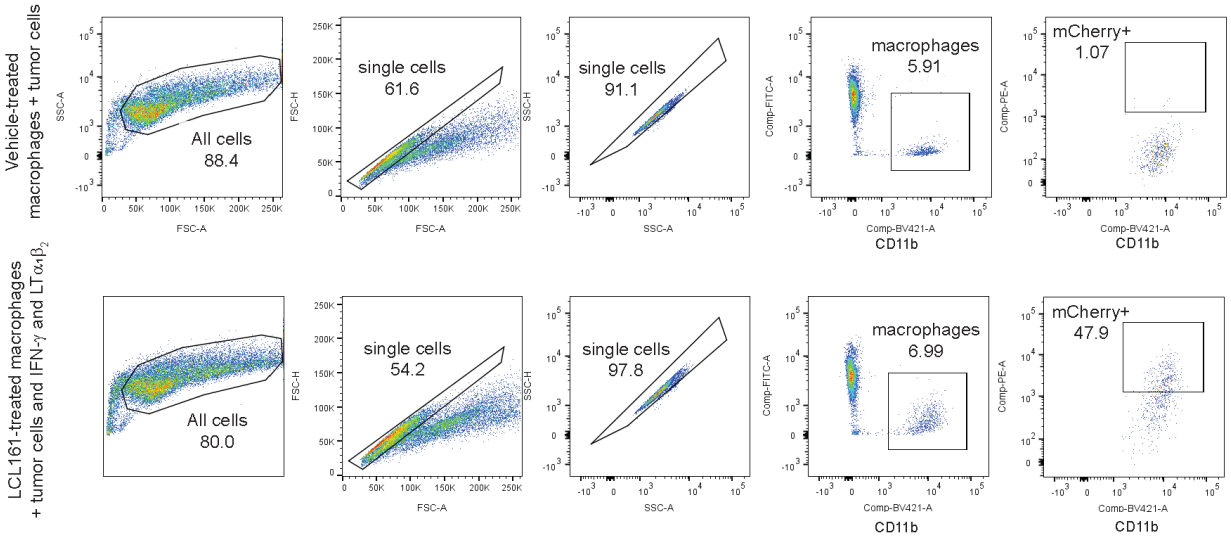

Fig. S8. Gating strategy for in vitro phagocytosis assays. BMDMs were differentiated from mouse bone marrow by culturing in CSF1 in 12-well plates. On day 7, medium was removed and 6694c2-mCherry cells were plated onto macrophages at a 1:5 ratio. Phagocytosis was defined by flow cytometry of cells after 18 hour coculture. Flow cytometry gating scheme is shown. FSC, forward scatter; SSC, side scatter; A, area; H, height; FITC, fluorescein isothiocyanate; BV, brilliant violet; PE, phycoerythrin; BMDMs, bone marrow-derived macrophages.

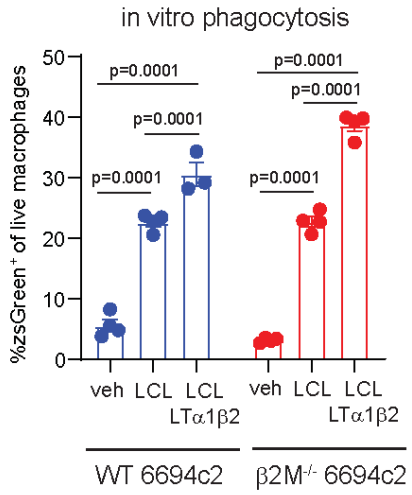

Fig. S9. MHC class I loss does not affect tumor cell susceptibility to phagocytosis. WT or $\beta 2M^{-/-}$ 6694c2 zsGreen+ cells were cultured with BMDMs that had been pretreated for 24 hours with 500nM LCL161 and lymphotoxin ($LT\alpha 1\beta 2$). Rates of phagocytic uptake were determined after 24 hours of coculture by flow cytometry. Data are representative of 3 independent experiments with 3-4 replicates per group. Data are presented as mean ± SD and p-values were calculated by ANOVA.

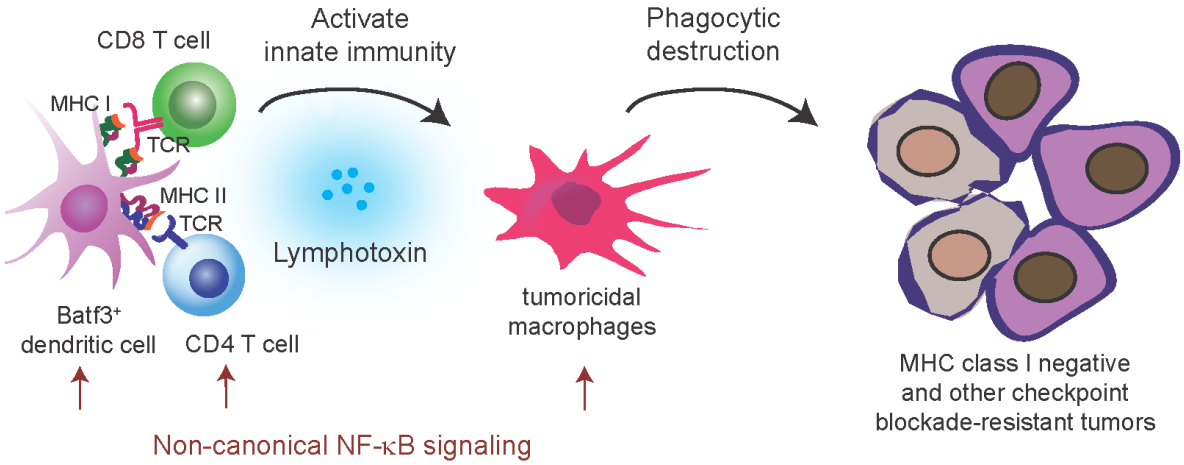

Fig. S10. Model of mechanism of action of cIAP1/2 antagonism in vivo in poorly immunogenic tumors.

Movie S1. Dynamics of cocultured macrophages and pancreatic cancer cells. BMDMs were plated onto glass-bottomed imaging dishes and cultured 24 hours, washed, and cocultured with 6694c2 tumor cells. Time lapse images were acquired for 12 hours at 2.5 min intervals in the presence of Sytox blue. BMDMs are capable of engulfing dead tumor cell debris as indicated by the pink arrows.

Movie S2. Engulfment of live tumor cells by tumoricidal macrophages. BMDMs were plated onto glass-bottomed imaging dishes and pretreated with LCL161, lymphotoxin, and IFN-γ for 24 hours before washing and coculturing with 6694c2 cells. Time lapse images were acquired for 12 hours at 2.5 min intervals in the presence of Sytox blue. Note the white arrows indicating BMDMs reaching and engulfing live tumor cells.